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Synthesis, Characterization and Antitubercular Evaluation of Novel Isatin Derivatives

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Abstract – Tuberculosis is caused by a bacterium called Mycobacterium tuberculosis, attacks the lungs but may also attack other parts of the body such as the kidney, spine, and brain. Tuberculosis may also be linked to certain risk factors, including alcoholism, IV drug abuse, and homelessness. Infection with Tubercle bacillus (most often M. tuberculosis) is characterized by the formation of tubercles.

Since the main antitubercular drugs have developed resistant to the large proportion of TB patients. Bacteria's are showing tolerances for more of antibiotics. The long treatment due to resistance causes heptotoxicity, nephrotoxicity etc. So there the Isatin was selected as template for modification and development of a compound library because anti-tubercular potential of novel Isatin derivatives like the first antitubercular potential of Isatin was confirmed by Erdman and Laurent in 1841. Isatin has medicinal potential to be used as drug template for identifying the novel drug candidates which can eliminate these treatments associated problems and can work potentially over first line drug resistant. The synthesized derivatives were potentially characterized for their purity through the chromatography and spectroscopic techniques.

The confirmed derivatives were tested, the results showed like the few compounds from Bromoisatin derivatives found to show 50% MTB inhibition at concentration between 20µM to 30µM. The compounds with hydrocarbon attached substituent could show little good activity that may be due the little enhanced liphophilicity of the compounds which made the entry of the compounds easy into the MTB.

The Pyrimidine derivatives of Bromoisatin were showed good results than the Bromoisatin derivatives due the significant medicinal effect of attached Pyrimidine ring which may have enhance the liphophilicity and metabolic resistance as well. Due that two compounds 32 and 33 could kill the 50% of MTB even at concentration lower than 20µM. The study led to the knowledge that the isatin has antitubercular value. The Isatin scaffold may be utilized as template for further modification and development of drug like candidate.

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INTRODUCTION

Tuberculosis is caused by a bacterium called *Mycobacterium tuberculosis*. This bacterium typically attacks the lungs but may also attack other parts of the body such as the kidney, spine, and brain. Tuberculosis may also be linked to certain risk factors, including alcoholism, IV drug abuse, and homelessness. Infection with *Tubercle bacillus* (most often *M. tuberculosis*) is characterized by the formation of tubercles, hard nodules in the lungs that are the result of interaction between the bacteria and the host's immune system. The infected macrophages result in an inflammatory response (heat, swelling, dilated capillaries) which attracts more macrophages until the site of infection is completely surrounded by many of these compressed phagocytic cells. Inflammation triggers other cells within the host to essentially quarantine the area by depositing collagen fibers around the packed macrophages, forming an enclosed infection within the lung called a tubercle. The cells at the center of the tubercle may eventually die, producing either an area of necrosis or an actual cavity. Tuberculosis usually attacks the lungs (pulmonary tuberculosis) but it also affects the central nervous system, the lymphatic system, the circulatory system, the genitourinary system, the gastrointestinal system, bones, joints, and even the skin **(Wehenkel, *et al.* 2008).**

Resistance is growing for standard anti-tubercular drugs have been used frequently. Disease strains

that are resistant to a single anti-TB drug have been documented in every country surveyed. Multidrug-resistant tuberculosis (MDR-TB) is a form of TB caused by bacteria that do not respond to, at least, isoniazid, ethambutol and rifampicin, the two most powerful, first-line (or standard) anti-TB drugs. About 450 000 people developed MDR-TB in the world in 2012. More than half of these cases were in India, China and the Russian Federation. It is estimated that about 9.6% of MDR-TB cases had XDR-TB. **(WHO Fact sheet 2019,** http://www.who.int/mediacentre/factsheets/fs104/en/) .

Disease caused by resistant bacteria fails to respond to conventional, first-line treatment. MDR-TB is being treated by using second-line drugs but there is no satisfactory results have been found. However second-line treatment options are limited and recommended medicines are not always available. The extensive chemotherapy required (up to two years of treatment) is more costly and can produce severe adverse drug reactions in patients. Isoniazid, rifampicin and ethambutol main first line antitubercular drugs have been found not effective for resistant mycobacterium tuberculosis individually or not in combination therapy. Isoniazid has been experimentally identified resistant towards tuberculosis. Isoniazid is also responsible for heptotoxicity at the cost of long term treatment. Rifampicin is also a first line drug that has been found not effective against mutated mycobacterium tuberculosis (MTB) in combination drug therapy. Rifampicin has become resistant to kill MTB pathogen.

Because INH is the most commonly used antitubercular drug, resistance to INH occurs more frequently among clinical isolates than resistance to any other agent **(Karakousis, 2009).** Mutations have been commonly detected in the *katG* gene in INH-resistant clinical isolates occurring in 50–80% of cases. Mutations in *katG* gene reduce or affect the ability of the catalase-peroxidase to activate the INH pro-drug. Commonly point mutations in *katG* are observed and a single point mutation which is responsible for substitution of threonine for serine at residue 315 (S315T) accounts for the majority of INH resistance among clinical isolates **(Marttila, *et al.* 1998; Abate, *et al.* 2001).** INH resistance has also been found to arise from mutations in *inhA* that can also result in reduced affinity of the enzyme for NADH without affecting enoyl reductase activity of NADH **(Basso, *et al.* 1998).** Mutations in *inhA* also have been found to cause resistance to the structurally related second-line drug ethionamide.

The rifamycins were first isolated in 1957 from *Amycolatopsis mediterranei* as part of an Italian antibiotic screening program **(Sensi, 1983).** While INH resistance alone is more common in *M. tuberculosis* than resistance to rifampin alone and more than 90% of rifampin-resistant isolates has been found also resistant to INH. Therefore, rifampin resistance isolates has been used as a substitute marker for MDR-TB. Resistance to rifampin in *M. tuberculosis* is caused most commonly as single point mutations in the *rpoB* gene, which encodes the RNA polymerase **(Telenti, *et al.* 1993).** Point mutations cluster in an 81-base pair “hot-spot” region between codons 507 and 533 of the *rpoB* gene, with mutations in codons 531 encodes Serine and codons 526 encodes Histidine predominatly in More than 90% of rifampin-resistant clinical isolates **(Ramaswamy and Musser 1998).**

Duration of treatment vital to achieve acceptable relapse rates has been reduced to six months from 9–12 months since the discovery of pyrazinamide (PZA) **(Steele and Des Prez 1988).** PZA resistance has been recognized primarily due to mutations in the *pncA* gene which encods PZase **(Scorpio and Zhang 1996).** Most of mutations found are due to point mutations, deletions, and insertions which have been reported in a 561-bp region of the open reading frame or in an 82-bp region of its putative promoter **(Scorpio, *et al.* 1997; Jureen, *et al.* 2008).** A small percentage of isolates with high-level PZA resistance contain no mutations in *pncA* or its promoter that suggests about alternative mechanisms of resistance such as deficient uptake and enhanced efflux and altered *pncA* regulation **(Raynaud, *et al.* 1999).**

Resistance to ethambutol in *M. tuberculosis* is commonly found to be caused due to point mutations in the *embCAB* operon **(Belanger, *et al.* 1996).** The *EmbA* and *EmbB* proteins are found to involve in the formation of the proper terminal hexaarabinofuranoside motif during arabinogalactan synthesis **(Escuyer, *et al.* 2001)** where *EmbC* is found to involve in lipoarabinomannan synthesis **(Zhang, *et al.* 2003).** *EmbB* is considered to be the main target of ethambutol because more percentage of EMB-resistant clinical isolates found to have mutations in the *embB* gene **(Sreevatsan, *et al.* 1997; Telenti, *et al.* 1997; Ramaswamy, *et al.* 2000).**

Antibiotic tolerance is the capability of non-replicating bacteria to get resistant againt particular antibiotic i.e the bacteria resist killing by cell wall-active antibiotics **(Tomasz, *et al.*1970).** This occurrence of tolerance is distinct from drug resistance as that can be intrinsic or acquired tolerance since it is not attributable to genetic mutations, and the organisms regain susceptibility to these antibiotics once the stress conditions have been removed and bacterial growth resumes. The prolonged treatment with antibiotics required to eradicate TB is supposed to alter the physiological state of persistent bacilli which have developed tolerance to standard anti-tuberculosis drugs, particularly to isoniazid, which inhibits mycolic acid synthesis **(Karakousis, *et al.* 2008; Adhikari, 2010).**

METHODOLOGY

Synthesis of Isatin derivatives

The chalcone and pyrimidine derivatives of Isatin as Series-I and Series-II were synthesized and characterized by recording IR, ^{1}HNMR, ^{13}C NMR and MASS spectra as published in our previous research paper **(Ramesh Kumar and Mahesh Kumar 2019; Ramesh Kumar and Mahesh Kumar 2018)** .

Anti-tubercular activity

All the synthesized were tested *in-vitro* for anti-tubercular activity. Alamar blue susceptibility test (MABA): Antimicrobial susceptibility testing will be performed in black, clear-bottomed, 96-well microplates (black view plates; Packard Instrument Company, Meriden, Conn.) in order to minimize background fluorescence. Outer perimeter wells will be filled with sterile water to prevent dehydration in experimental wells. **(Collins and Franzblau 1997).** The M. tuberculosis (RCMB 010126) strain was used for anti-tubercular activity and the reference drugs Isoniazide and pyrazinamide were used. There the anti MTB activity was of the derivatives was done based on the Alamar blue assay (MABA). Assay was done in black, clear-bottomed, 96 well microplates. The serial dilutions of the each derivative were made for the testing. The plates containing MTB and test compounds were incubated at 37 °C. The compounds were tested in triplicates. The IC_{50} of the derivatives was calculated.

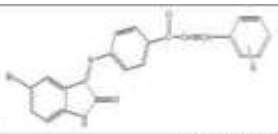
RESULTS

Compound library enumeration and Druglikeness prediction

The compound library developed was screened for their druglikeness using the online available tool DataWarriar. The compounds were filtered through Lipinski rule of five which decides the physicochemical properties of the compounds needed for being a druglike compound. The compounds were found to possess druglike properties except comp. 3j P-Dimethylamino substituted analog which showed high risk of carcinogenicity and mutagenicity. The comp. 3c, 4-OCH_3 substituted analog and 3j also showed little probability of reproductive effects as shown in Table (1 & 2).

Chalcone derivatives of 5-Bromoisatin

Table.1: The substituent (R) groups for the compound designing (Series-I)



Substituent's (R)			
3a	H	**3i**	2,4-Cl
3b	2-OCH_3	**3j**	P-Dimethylamino
3c	4-OCH_3	**3k**	2-Cl
3d	4-OH	**3l**	2-Br
3e	4-Cl	**3m**	3-NO_2
3f	4-Br	**3n**	3,4-OCH_3
3g	2-NO_2	**3o**	4-OH, 3OCH_3
3h	4-NO_2		

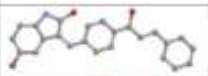
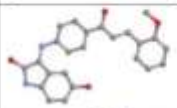
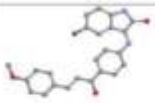
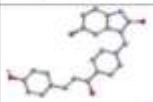
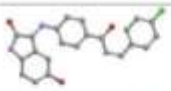
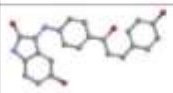
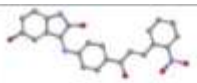
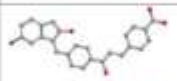
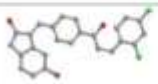
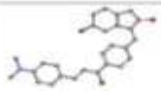
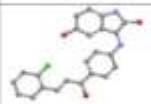
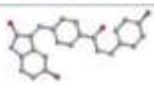
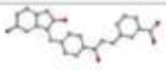
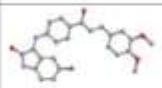
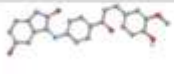
Table.2: Physicochemical parameters of compounds as per Lipisnki rule of five.

Comp.Id	HB A	HBD	cLogP	MW	PSA	LRV	M	C	RE
3a	4	1	4.028	431.288	58.53	0	False	False	False
3b	5	1	3.958	461.314	67.76	0	False	False	False
3c	5	1	3.958	461.314	67.76	0	False	False	**low**
3d	5	2	3.6823	447.287	78.76	0	False	False	False
3e	4	1	4.634	465.733	58.53	0	False	False	False
3f	4	1	4.7532	510.184	58.53	**1**	False	False	False
3g	7	1	3.1064	476.285	104.35	0	False	False	False
3h	7	1	3.1064	476.285	104.35	0	False	False	False
3i	4	1	5.24	500.178	58.53	**2**	False	False	False
3j	**5**	**1**	**3.9244**	**474.357**	**61.77**	**0**	**High**	**High**	**low**
3k	4	1	4.634	465.733	58.53	0	False	False	False
3l	4	1	4.7532	510.184	58.53	**1**	False	False	False
3m	7	1	3.1064	476.285	104.35	0	False	False	False
3n	6	1	3.888	491.340	76.99	0	False	False	False
3o	6	2	3.61235	477.313	87.99	0	False	False	False

HBA: Hydrogen bond acceptor; **HBD:** Hydrogen bond donor; **MW:** Molecular weight; **PSA:** Polar surface area; **LRV:** Lipinski rule violations; **M:** Mutagenic; **C:** Carcinogenic; **RE:** Reproductive effects.

A series Isatin derivative was synthesized. A significant number of derivatives showed potential anti-TB activities with IC_{50} in a range of 20.2 to 58.0 µM concentration. The IC50 was found very high than the references drugs Pyrazinamide and INH (IC50: 0.083µg/mL and 0.105µg/mL). The derivatives containing methoxy substituent showed the better inhibition (50% inhibition) of MTB growth below 30 µM.

Table.4: 50% inhibition of MTB parasite at incubation with the synthesized compounds.

S.No.	Comp. Id	Structure	IUPAC Name	IC_{50} (µM)
1.	3a		(3Z)-5-bromo-3-{[4-[(2E)-3-phenylprop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	32.1
2.	3b		(3Z)-5-bromo-3-{[4-[(2E)-3-(2-methoxyphenyl)prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	20.2
3.	3c		(3Z)-5-bromo-3-{[4-[(2E)-3-(4-methoxyphenyl)prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	22.1
4.	3d		(3Z)-5-bromo-3-{[4-[(2E)-3-(4-hydroxyphenyl)prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	35.3
5.	3e		(3Z)-5-bromo-3-{[4-[(2E)-3-(4-chlorophenyl)prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	46.2
6.	3f		(3Z)-5-bromo-3-{[4-[(2E)-3-(4-bromophenyl)prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	58.0
7.	3g		(3Z)-5-bromo-3-{[4-[(2E)-3-(2-nitrophenyl)prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	28.7
8.	3h		(3Z)-5-bromo-3-{[4-[(2E)-3-(4-nitrophenyl)prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	30.2
9.	3i		(3Z)-5-bromo-3-{[4-[(2E)-3-(2,4-dichlorophenyl]prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	49.7
10.	3j		CN(C)c1ccc(cc1)/C=C/C(=O)c1ccc(cc1)/N=C1/c2cc(Br)ccc2NC1=O	34.1
11.	3k		(3Z)-5-bromo-3-{[4-[(2E)-3-(2-chlorophenyl]prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	51.2
12.	3l		(3Z)-5-bromo-3-{[4-[(2E)-3-(4-bromophenyl]prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	28.6
13.	3m		(3Z)-5-bromo-3-{[4-[(2E)-3-(3-nitrophenyl]prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	36.7
14.	3n		COc1ccc(cc1OC)/C=C/C(=O)c1ccc(cc1)/N=C1/c2cc(Br)ccc2NC1=O	32.6
15.	3o		(3Z)-5-bromo-3-{[4-[(2E)-3-(4-hydroxy-3-methoxyphenyl]prop-2-enoyl]phenyl]imino}-1,3-dihydro-2*H*-indol-2-one	25.3
16.		Isoniazide	Reference drug	0.105 µg/mL
17.		Pyrazinamide	Reference drug	0.83 µg/mL

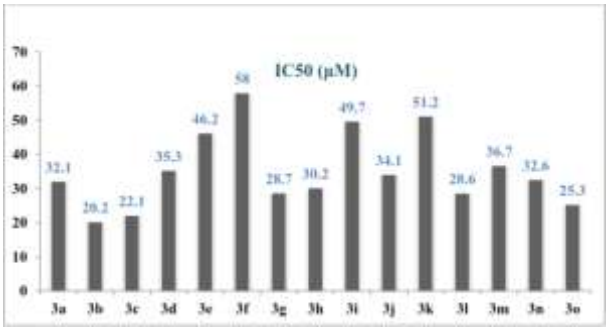
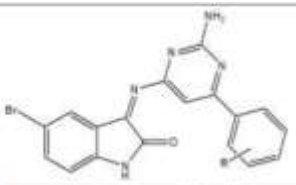


Figure.1: IC_{50} of chalcone derivatives of 5-Bromoisatin.

Activity of Pyrimidine derivative of 5-Bromoisatin

The Pyrimidine derivatives showed little better antitubercular activity. There derivatives 32 and 33 were found to show the 50% inhibition of MTB bacteria at the concentration below the 20 micromolar of concentration. The other twelve compound derivatives also showed the IC_{50} below the 50µM except 36th compound. The activity of the derivatives was found very far higher than the reference drugs.

Table.5: The substituent (R) groups for the compound designing



Substituent (R)

Compounds	-R	Compounds	-R
31	-H	39	2,4-Cl
32	2-OCH_3	40	*p*-Dimethylamino
33	4-OCH_3	41	2-Cl
34	4-OH	42	2-Br
35	4-Cl	43	3-NO_2
36	4-Br	44	3,4-OCH_3
37	2-NO_2	45	4-OH,3-OCH_3
38	4-NO_2		

Table.6: Physicochemical parameters of compounds as per Lipisnki rule of five.

Comp.Id	HB A	HBD	cLogP	MW	PSA	LRV	M	C	RE
3a	4	1	4.028	431.288	58.53	0	False	False	False
3b	5	1	3.958	461.314	67.76	0	False	False	False
3c	5	1	3.958	461.314	67.76	0	False	False	**low**
3d	5	2	3.6823	447.287	78.76	0	False	False	False
3e	4	1	4.634	465.733	58.53	0	False	False	False
3f	4	1	4.7532	510.184	58.53	**1**	False	False	False
3g	7	1	3.1064	476.285	104.35	0	False	False	False
3h	7	1	3.1064	476.285	104.35	0	False	False	False
3i	4	1	5.24	500.178	58.53	**2**	False	False	False
3j	**5**	**1**	**3.9244**	**474.357**	**61.77**	**0**	**High**	**High**	**low**
3k	4	1	4.634	465.733	58.53	0	False	False	False
3l	4	1	4.7532	510.184	58.53	**1**	False	False	False
3m	7	1	3.1064	476.285	104.35	0	False	False	False
3n	6	1	3.888	491.340	76.99	0	False	False	False
3o	6	2	3.61235	477.313	87.99	0	False	False	False

HBA: Hydrogen bond acceptor; **HBD:** Hydrogen bond donor; **MW:** Molecular weight; **PSA:** Polar surface area; **LRV:** Lipinski rule violations; **M:**

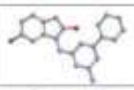
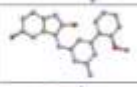
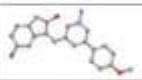
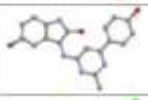
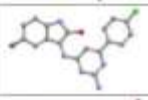
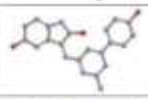
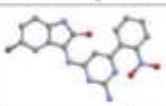
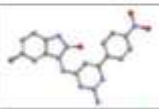
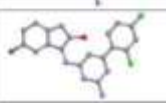
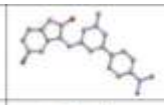
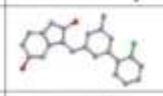
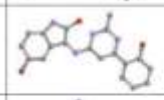
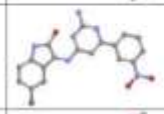
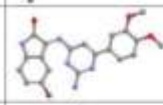
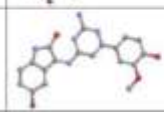
Mutagenic; **C:** Carcinogenic; **RE:** Reproductive effects.

Table.7: Physicochemical properties of synthesized compounds (31-45).

Compounds	Molecular Formula	Mol. Weight	Melting Point (°c)	Rf value	% Yield
31	$C_{10}H_{12}BrN_5O$	394.22	190-193	0.68	70.0
32	$C_{19}H_{14}BrN_5O_2$	424.25	212-214	0.71	68.0
33	$C_{19}H_{14}BrN_5O_2$	424.25	206-209	0.76	65.0
34	$C_{18}H_{12}BrN_5O_2$	410.22	196-199	0.65	71.0
35	$C_{18}H_{11}BrClN_5O$	428.67	230-233	0.69	76.0
36	$C_{10}H_{11}Br_2N_5O$	473.12	224-226	0.73	71.0
37	$C_{18}H_{11}BrN_6O_3$	439.22	228-230	0.77	64.0
38	$C_{18}H_{11}BrN_6O_3$	439.22	226-228	0.75	69.0
39	$C_{18}H_{10}BrCl_2N_5O$	463.11	218-221	0.65	61.0
40	$C_{20}H_{17}BrN_6O$	437.27	202-205	0.78	74.0
41	$C_{18}H_{11}BrClN_5O$	428.67	226-229	0.67	70.0
42	$C_{18}H_{11}Br2N_5O$	473.12	228-230	0.72	75.0
43	$C_{18}H_{11}BrN_6O_4$	455.22	222-224	0.74	68.0
44	$C_{20}H_{16}BrN_5O_3$	454.28	237-239	0.60	78.0
45	$C_{19}H_{14}BrN_5O_3$	440.25	234-236	0.64	80.0

(Solvent front: chloroform: benzene: acetic acid)

Table.8: 50% inhibition of MTB parasite at incubation with the synthesized compounds.

S.No.	Structure	IUPAC Name	IC_{50} (µM)
31		(3Z)-3-{[2-amino-6-phenylpyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	22.3
32		(3Z)-3-{[2-amino-6-(2-methoxyphenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	12.4
33		(3Z)-3-{[2-amino-6-(4-methoxyphenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	16.7
34		(3Z)-3-{[2-amino-6-(4-hydroxyphenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	31.5
35		(3Z)-3-{[2-amino-6-(4-chlorophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	41.1
36		(3Z)-3-{[2-amino-6-(4-bromophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	51.6
37		(3Z)-3-{[2-amino-6-(2-nitrophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	20.9
38		(3Z)-3-{[2-amino-6-(4-nitrophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	20.9
39		(3Z)-3-{[2-amino-6-(2,4-dichlorophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	41.2
40		(3Z)-3-{[2-amino-6-[4-(dimethylamino)phenyl]pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	32.4
41		(3Z)-3-{[2-amino-6-(2-chlorophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	46.1
42		(3Z)-3-{[2-amino-6-(2-bromophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	22.5
43		(3Z)-3-{[2-amino-6-(3-nitrophenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	33.6
44		(3Z)-3-{[2-amino-6-(3,4-dimethoxyphenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	24.7
45		(3Z)-3-{[2-amino-6-(4-hydroxy-3-methoxyphenyl)pyrimidin-4-yl]imino}-5-bromo-1,3-dihydro-2*H*-indol-2-one	22.8

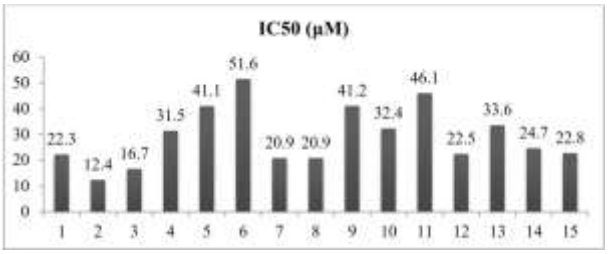


Figure.2: IC_{50} of Pyrimidine derivative of 5-Bromoisatin (Series-II).

CONCLUSION

The Isatin was selected as template for modification and development of a compound library because anti-tubercular potential of novel Isatin derivatives like the first antitubercular potential of Isatin was confirmed by Erdman and Laurent in 1841. Isatin has medicinal potential to be used as drug template for identifying the novel drug candidates which can eliminate these treatments associated problems and can work potentially over first line drug resistant. The synthesized derivatives were potentially characterized for their purity through the chromatography and spectroscopic techniques.

The confirmed derivatives were tested, the results showed like the few compounds from Bromoisatin derivatives found to show 50% MTB inhibition at concentration between 20µM to 30µM. The compounds with hydrocarbon attached substituents could show little good activity that may be due the little enhanced liphophilicity of the compounds which made the entry of the compounds easy into the MTB.

The Pyrimidine derivatives of Bromoisatin were showed good results than the Bromoisatin derivatives due the significant medicinal effect of attached Pyrimidine ring which may have enhance the liphophilicity and metabolic resistance as well.

Due that two compounds 32 and 33 could kill the 50% of MTB even at concentration lower than 20µM. The study led to the knowledge that the isatin has antitubercular value. The Isatin scaffold may be utilized as template for further modification and development of drug like candidate.

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